Inhibition of Akt/protein kinase B activity sensitizes moderately- to un-differentiated gastric cancer cells to chemotherapy

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Gastric cancer is the most common malignant tumor of the digestive tract in China. Development of resistance to chemotherapy also occurs frequently in tumor cells. Akt, also known as protein kinase B (PKB), is a 60kD serine/threonine kinase and functions downstream of phosphatidylinositol 3-kinase (PI-3K), controlling diverse cellular functions, including cell apoptosis, proliferation, differentiation, and glucose metabolism.[1] In the present study, we studied the activities of Akt/PKB in gastric cell lines with different differentiation degrees (MKN-28, well-differentiated; SGC-7901, moderately-differentiated; BGC-823, poorly-differentiated; HGC-27, undifferentiated), and explored the effects of Akt/PKB inhibition on cell survival rates and apoptosis rates with treatment of etoposide.

Cell culture. The current study was conducted between September 2005 and June 2006. The MKN-28 (well-differentiated), SGC-7901 (well-differentiated), BGC-823 (poorly-differentiated), and HGC-27 (undifferentiated) cells were cultured in RPMI1640 medium supplemented with 10% fetal bovine serum, 100U/ml streptomycin, 100U/ml penicillin in a humidified 5% CO_2 atmosphere at 37°C. The cells were digested with 0.25% trypsin and 0.02% EDTA every 2-3 days, and passed at ratio of 1:3-1:5.

Cell survival rate assay. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide colorimetric assay was used to determine the cell survival rate. Gastric cancer cells in the logarithmic phase were seeded in 96-well plates, and treated with 20μmol/L etoposide in the presence or absence of a 2 hour pretreatment of 40nmol/L wortmannin for 0 hours, 3 hours, 6 hours, 12 hours, and 24 hours. Cell survival rates were assayed at the above time points. When exposed to 20μmol/L etoposide within 24 hours, the 4 gastric cancer cell lines exhibited time-dependent inhibition of cell survival rates. Pretreatment with wortmannin potentiated the inhibitory effect of etoposide on cell survival rates in a time-dependent manner. Except for MKN-28, there was a significant decrease in cell survival rates in other cell lines with pretreatment of wortmannin compared to without (p<0.01).

Measurement of cell apoptosis rate. Cell apoptosis rate was determined with flow cytometry using double staining of annexin V and propidium iodide (PI). Gastric cancer cells were treated with 20μmol/L etoposide in the presence or absence of 2 hour pretreatment of 40nmol/L wortmannin for 0 hours, 3 hours, 6 hours, 12 hours, and 24 hours, followed by washing 2 times with 0.01mmol/L phosphate buffer solution. The cells were suspended with binding buffer and adjusted to a density of $1x10^6$/ml. Cell suspension (1 ml) was centrifuged at 200g for 5 minutes. The supernatant was then discarded. Cell pellets were resuspended in 80μl binding buffer, and incubated with 10μl annexin-V-fluorescein and 10μl PI solutions at room temperature in the dark for 10 minutes. After centrifugation and resuspension in binding buffer, the samples were analyzed by flow cytometry with excitation wavelength of 488nm. When treated with 20μmol/L etoposide within 24 hours, the 4 gastric cancer cell lines showed time-dependent elevation of cell apoptosis rates. Pretreatment with wortmannin enhanced the effect of etoposide on cell apoptosis rates in a time-dependent manner. The cell apoptosis rates were significantly increased in SGC-7901, BGC-823m and HGC-27 with pretreatment of wortmannin compared to without (p<0.01).

Determination of Akt/PKB activity. The Akt/PKB activity was determined using a non-radioactive protein kinase assay kit. Basal PKB activity was measured as follows. Gastric cancer cells in the logarithmic phase were digested, and total protein was extracted from the cell lysates. The total protein was then incubated with immobilized antibody specific for phospho-Akt/PKB to immunoprecipitate Akt/PKB. The latter was used to phosphorylate a specific substrate, GSK-3 fusion protein (GSK-3α/β). Phospho-GSK-3α/β was analyzed by western blot with its monoclonal antibody. Enhanced chemiluminescence (ECL) was used to detect phospho- GSK-3α/β, which indirectly reflects the activity of Akt/PKB. The Akt/PKB activities in gastric cancer cells treated with etoposide for 0 hours, 3 hours, 6 hours, 12 hours, and 24 hours were also measured with the above method. Non-radioactive protein kinase assay revealed that the basal activities of Akt/PKB in these cancer cell lines are in the contrary order of differentiation degrees, MKN-28 < SGC-7901 < BGC-823 < HGC-27. Treatment with 20μmol/L etoposide led to time-dependent induction in Akt/PKB activities within 24 hours in the 4 cancer cell lines. The Akt/PKB activity was undetectable in cell lines pretreated with wortmannin.

Discussion. Gastric cancer is the most common malignant tumor of the digestive tract in China,

and accounts for the second leading cause of death among malignant tumors. Surgery is the mainstay of treatment for gastric cancer. As an adjuvant therapy after surgical resection, chemotherapy has received considerable attention in the treatment of gastric cancer. However, development of insensitivity or resistance to chemotherapy occurs frequently in tumor cells.[2] Novel agents are, therefore, needed to sensitize the resistant cancer cells. Akt is a 60kD serine/threonine kinase and functions downstream of phosphatidylinositol 3-kinase (PI-3K). Activation of PI-3K generates phosphatidylinositol 3,4-bisphosphate, which may induce the membrane translocation of Akt coincident with its phosphorylation and activation. Akt is activated in response to insulin and growth factors, and upon activation, it phosphorylates several substrates including glycogen synthetase kinase-3 (GSK-3), Bax, Caspase-9. Activation of Akt kinase activity is inhibited by wortmannin or LY294002, inhibitors of PI-3K.[3] Over activation of Akt has been demonstrated in gastric cancer, and is correlated with clinicopathological parameters and poor outcome.[4] In our study, we determined the basal activities of Akt/PKB in 4 gastric cancer cell lines with different differentiation degrees, and found that Akt/PKB activity was inversely correlated with the degrees of differentiation of cancer cells, which may account for the reason why over activation of Akt is associated with poor outcome in gastric cancers.

Akt/PKB plays an important role in cell survival.[5] In our study, treatment with etoposide within 24 hours resulted in time-dependent inhibition of cell survival rates and induction of cell apoptosis rates in cancer cell lines. However, there was a marked induction of Akt/PKB activity in a time-dependent manner during the course of etoposide treatment. The induction of Akt/PKB activity may confer protection against etoposide-induced death because inhibition of Akt/PKB by wortmannin enhanced the effects of etoposide. Cell survival rates and apoptosis rates were significantly decreased and elevated respectively in SGC-7901, BGC-823, and HGC-27 cell lines after treatment with etoposide + wortmannin compared with etoposide alone. However, we found that the chemotherapy-sensitizing effect of wortmannin was related to the degree of differentiation of cancer cells. There was no significant difference in cell survival rates and apoptosis rates between treatments of etoposide + wortmannin and etoposide alone in MKN-28, a well-differentiated cell line. The MKN-28 had less activity of Akt/PKB than other cell lines, which may explain the lack of chemotherapy-sensitizing effect by wortmannin.

In conclusion, Akt/PKB activity is inversely correlated with the degree of differentiation of gastric cancer cells, and inhibition of Akt/PKB activity may sensitize moderately- to undifferentiated cancer cells to chemotherapy, which maybe extrapolated to clinical practice in selecting the patients for combined chemotherapy with Akt/PKB inhibitor.

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Random amplified polymorphic DNA typing of nosocomial *Candida albicans* isolates

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The incidence of candidiasis is dramatically on the rise due to the increase in the immuno-suppressed population, especially related to HIV infection, chemotherapy, and organ transplantation.[1] *Candida albicans* is the most frequent pathogenic species of candidiasis, and the mortality rate in candidiasis varies from 38-50%.[2,3] Because of its clinical importance, several typing schemes were developed to assess the strain identity. These typing methods are generally considered too variable to be of any practical value in epidemiological investigations.[4] With the advent of molecular genetics,